

Claims

1. A method of screening a translational fusion partner (TFP) for producing a non-producible protein, comprising:

5 (1) preparing an automatic screening vector including a fusion gene (X-R) in which a gene (X) encoding a non-producible target protein is linked in frame to a reporter gene (R) for automatic screening;

10 (2) linking a gene library including a TFP inducing secretion of the non-producible fusion protein (X-R) to the automatic screening vector to create a TFP library;

15 (3) transforming cells having no activity of the reporter gene with the TFP library to detect the activity of a reporter protein; and

(4) isolating a gene from transformed cells exerting the activity of the reporter protein and analyzing properties of the TFP.

2. The method according to claim 1, wherein the reporter gene is a gene selected from invertase, amylase, glucoamylase, galactosidase, sucrase, cellulase, xylanase and maltase.

3. The method according to claim 1, wherein the non-

producible protein is selected from cytokines, serum proteins, immunoglobulins, interferons, colony stimulating factors, stem cell factor (SCF), phospholipase activating protein (PLAP), insulin, tumor necrosis factor (TNF),
5 growth factors, lactoferrin, hormones, calcitonin, calcitonin gene related peptide (CGRP), enkephalin, somatomedin, erythropoietin, hypothalamic releasing factor, prolactin, chorionic gonadotropin, tissue plasminogen activator, growth hormone releasing peptide (GHRP), thymic
10 humoral factor (THF), anticancer or antibiotic peptides, carbohydrate-specific enzymes, proteolytic enzymes, lipases, oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases.

4. The method according to claim 1, wherein the gene library with a TFP is derived from *Candida*, *Debaryomyces*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Schizosaccharomyces*, *Yarrowia*, *Saccharomyces*, *Aspergillus*, *Penicillium*, *Rhizopus* and *Trichoderma*.

5. The method according to claim 1, wherein the cells having no activity of the reporter gene are selected from *Candida*, *Debaryomyces*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Schizosaccharomyces*, *Yarrowia*, *Saccharomyces*, *Aspergillus*, *Penicillium*, *Rhizopus* and *Trichoderma*.

6. The method according to claim 1, wherein the screening vector comprises a promoter gene, a gene encoding a target protein, which is deleted for translation initiation and termination codons, and a reporter gene
5 fused in frame to the gene encoding the target protein.

7. The method according to claim 1, wherein the promoter contained in the screening vector is selected from *GAPDH*, *PGK*, *ADH*, *PHO5*, *GAL1* and *GAL10*.

8. A method of screening a translational fusion
10 partner, comprising:

- (1) preparing a yeast mutant strain deleted for its endogenous invertase gene *INV2(I)*;
- (2) preparing yeast high-throughput selection (HTS) vectors containing a gene (*X-I*) in which an invertase gene (*I*) is fused in frame to a non-producible protein gene (*X*) and which is controlled in expression under a yeast *GAL10* promoter;
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- (3) preparing a translational fusion partner library from yeast genes capable of secreting the fusion gene (*X-I*) of an invertase and a non-producible protein in the pYHTS vectors;
- (4) transforming the library into the yeast mutant strain prepared at step (1) and performing automatic screening on a medium containing only
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sucrose as a carbon source;

(5) detecting a protein secreted into the medium by culturing yeast cells grown on the sucrose medium; and

5 (6) isolating genes from the yeast cells and analyzing properties of the translational fusion partner.

9. A translational fusion partner (TFP) protein selected from among:

10 (a) a translational fusion partner TFP1 protein having an amino acid sequence represented by SEQ ID NO. 1 or an amino acid sequence 90% or higher homology thereto and exerting translational fusion partner TFP1 protein activity;

15 (b) a translational fusion partner TFP2 protein having an amino acid sequence represented by SEQ ID NO. 3 or an amino acid sequence 90% or higher homology thereto and exerting translational fusion partner TFP2 protein activity;

20 (c) a translational fusion partner TFP3 protein having an amino acid sequence represented by SEQ ID NO. 5 or an amino acid sequence 90% or higher homology thereto and exerting translational fusion partner TFP3 protein activity;

25 (d) a translational fusion partner TFP4 protein

having an amino acid sequence represented by SEQ ID NO. 7 or an amino acid sequence 90% or higher homology thereto and exerting translational fusion partner TFP4 protein activity;

5 (e) a translational fusion partner TFP1-3 protein having an amino acid sequence represented by SEQ ID NO. 9 or an amino acid sequence 90% or higher homology thereto and exerting translational fusion partner TFP1-3 protein activity;

10 (f) a translational fusion partner TFP1-4 protein having an amino acid sequence represented by SEQ ID NO. 10 or an amino acid sequence 90% or higher homology thereto and exerting translational fusion partner TFP1-4 protein activity;

15 (g) a translational fusion partner TFP4 protein having an amino acid sequence represented by SEQ ID NO. 40 or an amino acid sequence 90% or higher homology thereto and exerting translational fusion partner TFP3-1-1 protein activity; and

20 (h) a translational fusion partner TFP4 protein having an amino acid sequence represented by SEQ ID NO. 42 or an amino acid sequence 90% or higher homology thereto and exerting translational fusion partner TFP3-1-2 protein activity.

25 10. A translational fusion partner (TFP) gene

selected from among:

- (a) DNA encoding a translational fusion partner TFP1 protein represented by SEQ ID NO. 1 or DNA 90% or higher homologous thereto and encoding a protein exerting translational fusion partner TFP1 protein activity;
- (b) DNA encoding a translational fusion partner TFP2 protein represented by SEQ ID NO. 3 or DNA 90% or higher homologous thereto and encoding a protein exerting translational fusion partner TFP2 protein activity;
- (c) DNA encoding a translational fusion partner TFP3 protein represented by SEQ ID NO. 5 or DNA 90% or higher homologous thereto and encoding a protein exerting translational fusion partner TFP3 protein activity;
- (d) DNA encoding a translational fusion partner TFP4 protein represented by SEQ ID NO. 7 or DNA 90% or higher homologous thereto and encoding a protein exerting translational fusion partner TFP4 protein activity;
- (e) DNA encoding a translational fusion partner TFP1-3 protein represented by SEQ ID NO. 9 or DNA 90% or higher homologous thereto and encoding a protein exerting translational fusion partner TFP1-3 protein activity;

(f) DNA represented by SEQ ID NO. 10 or DNA 90% or higher homologous thereto and encoding a protein exerting translational fusion partner TFP1-4 protein activity;

5 (g) DNA encoding a translational fusion partner TFP3-1-1 protein represented by SEQ ID NO. 40 or DNA 90% or higher homologous thereto and encoding a protein exerting translational fusion partner TFP3-1-1 protein activity; and

10 (h) DNA encoding a translational fusion partner TFP3-1-2 protein represented by SEQ ID NO. 42 or DNA 90% or higher homologous thereto and encoding a protein exerting translational fusion partner TFP3-1-2 protein activity.

15 11. The translational fusion partner (TFP) gene according to claim 10, which is selected from DNA of SEQ ID NOS. 2, 4, 6, 8, 41 and 43.

12. A recombinant vector comprising a gene selected from the DNA of claim 10 (a) to (h).

20 13. The recombinant vector according to claim 12, which comprises a gene selected from DNA of SEQ ID NOS. 2, 4, 6, 8, 41 and 43.

14. The vector according to claim 6, wherein the
vector is selected from pYIL-KRTFP1 (KCTC 10544BP), pYIL-
KRT1-3 (KCTC 10548BP), pYIL-KRT1-4 (KCTC 10549BP), pYIL-
KRTFP2 (KCTC 10545BP), pYIL-KRTFP3 (KCTC 10546BP), pYGT3-1-
5 1-GCSF (KCTC 10753BP), pYGT3-1-2-GCSF (KCTC 10754BP) and
pYIL-KRTFP4 (KCTC 10547BP).

15. A transformant transformed with the recombinant
vector of claim 12.

16. The transformant according to claim 15, which is
10 a yeast selected from *Candida*, *Debaryomyces*, *Hansenula*,
Kluyveromyces, *Pichia*, *Schizosaccharomyces*, *Yarrowia* and
Saccharomyces.

17. A method of preparing a non-producible protein,
comprising constructing an expression vector carrying a
15 gene encoding a non-producible protein, which is fused to a
gene encoding the translational fusion partner (TFP)
protein of claim 9, and culturing yeast cells transformed
with the recombinant expression vector.